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Biaryl isoxazolinone antibacterial agents

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Abstract—In an era of increasing resistance to classical antibacterial agents, the synthetic oxazolidinone series of antibiotics has attracted much interest. Zyvox™ was the first oxazolidinone to be approved for clinical use against infections caused by multi-drug resistant Gram-positive bacteria. In the course of studies directed toward the discovery of novel antibacterial agents, a new series of synthetic phenyl-isoxazolinone agents that displayed potent activity against Gram-positive bacterial strains was recently discovered at Bristol-Myers Squibb. Extensive investigation of various substitutions on the phenyl ring was then undertaken. We report here, the synthesis and antibacterial activity of a series of biaryl isoxazolinone compounds.

The development of bacterial resistance to current therapies is an important driving force behind the discovery of new antibiotics that function through novel mechanisms of action. The incidence of vancomycin-resistant *Enterococcus faecium* (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA) infections in intensive-care units of hospitals has significantly increased between 1989 and 1997.[1] A more troubling occurrence was the first case of vancomycin-resistant *S. aureus* (VRSA) infection.[2] The oxazolidinones, a new class of synthetic antibacterial agents, are active against a variety of clinically important susceptible and resistant Gram-positive organisms. Although their mode of action is not clearly understood, these compounds appear to inhibit protein synthesis at the initiation of translation by binding directly to the 50S ribosomal subunit.[3]

The first oxazolidinone was reported by Dupont (**1**, Dup-721, Fig. 1)[4] but was eventually shown to be toxic and development was discontinued. This was followed by the Pharmacia clinical candidates eperezolid (**2**) and linezolid (**3**, now marketed as Zyvox™).[3,5] Zyvox™ is currently being used for complicated and uncomplicated skin and soft tissue infections, community- and hospital-acquired pneumonia and drug-resistant Gram-positive infections (MRSA and VRE). Several recent compounds in preclinical development have been disclosed, including AZD2563[6] from AstraZeneca and RBX 7644 (Ranbezolid)[7] from Ranbaxy.

Linezolid possesses many attributes which make it an attractive starting point for the design of novel antibacterials with a similar mechanism of action. Our investigation has resulted in the discovery of the 4-arylisoxazolin-5-one (**4**)[8] as an oxazolidinone isostere. Consequently, this paper will outline the synthesis and antibacterial activity of a series of aryl and heteroaryl phenyl-isoxazolinones.

The preparation of the various aryl or heteroaryl phenyl-isoxazolinones is as follows. To facilitate analog preparation, two common intermediates were prepared that allowed for late stage derivatization. The diazonium

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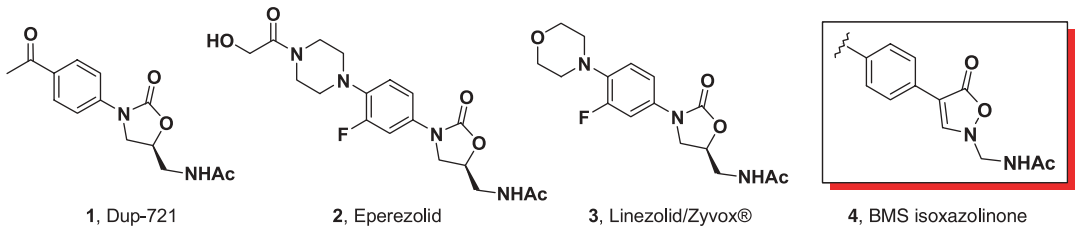
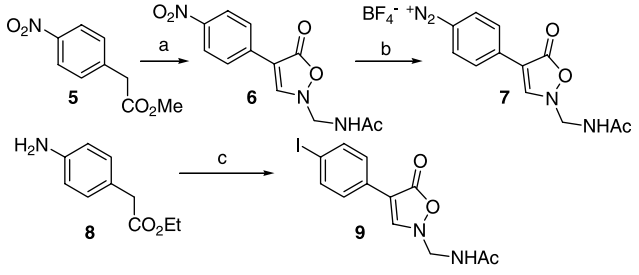


Figure 1. Early clinical oxazolidinones and BMS isoxazolinone antibacterial core.

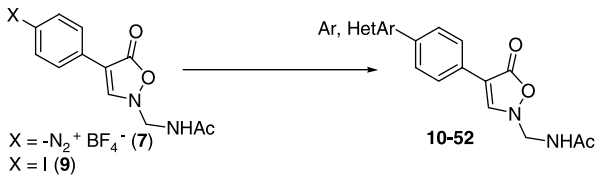
compound **7** and the iodo derivative **9**, that were used in a modified Suzuki reaction[9] or a Stille coupling employing Farina's method.[10] Briefly (Scheme 1), methyl-*p*-nitrophenyl acetate (**5**) was reacted with DMF–DMA and, subsequently, hydroxylamine to give the isoxazolinone core. After introduction of the acetamidomethyl side chain (**6**), the nitro functionality was reduced to the aniline which was then converted to the diazonium compound (**7**). Preparation of analogs via cross-coupling using a modified Suzuki reaction[9] with boronic acids were then carried out to introduce various substituents (Scheme 2). The preparation of the iodo derivative (**9**) started with ethyl-*p*-aminophenyl acetate (**8**). After diazotization and reaction with potassium iodide, the resulting acetate was treated with ethyl formate and hydroxylamine to afford the iodophenyl-isoxazolinone. Reaction with the acetamidoacetoxy-methyl electrophile gave intermediate **9** which was submitted to Stille coupling conditions employing Farina's method[10] (Scheme 2) to introduce various substituents.

Reduced analogs, for example the dihydropyran, dihydrothiopyran, and dehydropiperidines, were prepared starting with the corresponding vinyl stannanes (**54a–c**). These were easily prepared (Scheme 3) via addition of the tin anion onto ketones **53a–c** followed by elimination.[11] These stannanes were then used as the organometallic counterparts in Stille reactions with the iodo derivative **9**, giving **55a–c**. Functional group transformations provided the sulfoxide (**55d**), sulfone (**55e**), the deprotected dehydropiperidine (**55f**), as well as the acylated analogs (**55g–i**).

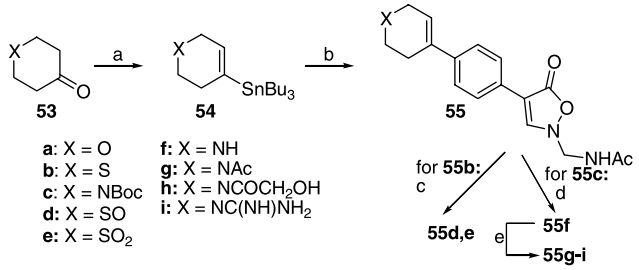
The reduced (tetrahydropyran and piperidine) analogs required the development of de novo syntheses as reduction of the vinyl-isoxazolinone derivatives failed. Consequently (Scheme 4), Suzuki cross-coupling[12] of vinyl triflates **57a,b** with pinacol arylboronate **56**[13] provided the desired analogs **58a,b**. Reduction at this stage followed by construction of the isoxazolinone was accomplished in standard fashion. Further elaboration provided the desired analogs (**60a–e**).



Scheme 1. Preparation of the isoxazolinone cross-coupling precursors. Reagents and conditions: (a) 1. DMF–DMA, 2. $H_2NOH{\cdot}H_2O$/EtOH/NEt_3, 3. H_2SO_4/$AcOCH_2NHAc$ (92%); (b) 1. H_2/Pd–C/MeOH (85%), 2. $NaNO_2$/HBF_4/H_2O/EtOH (72%); (c) 1. $NaNO_2$/KI/HCl/THF/H_2O (89%), 2. (i) NaH/EtOCHO, (ii) H_2NOH/MeOH (88%), 3. H_2SO_4/$AcOCH_2NHAc$ (72%).



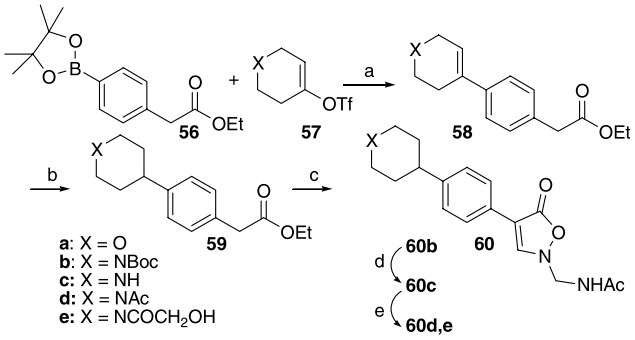
Scheme 2. Cross-coupling methodology for the preparation of isoxazolinone analogs. Reagents and conditions: For **7**: $ArB(OH)_2$/$Pd(OAc)_2$/MeOH, 50 °C; For **9**: $ArSnBu_3$/$Pd_2(dba)_3$/$AsPh_3$/NMP/70 °C.



Scheme 3. Synthesis of reduced heterocyclic analogs. Reagents and conditions: (a) 1. Bu_3SnLi/THF/−78 °C, 2. MsCl/Et_3N/CH_2Cl_2: 36% for **54a**, 78% for **54b**, 59% for **54c**; (b) **9**/$Pd_2(dba)_3$/$AsPh_3$/NMP/70 °C: 70% for **55a**, 53% for **55b**, 57% for **55c**; (c) for X = S: 1 equiv CH_3CO_3H/MeOH/CH_2Cl_2 gave **55d** (73%); excess CH_3CO_3H gave **55e** (77%); (d) For X = NBoc: TFA/CH_2Cl_2: 93% for **55f**; (e) For **55g**: Ac_2O/Py/0 °C (63%). For **55h**: 1. $TBSOCH_2COCl$/Et_3N/DCM/DMF (65%), 2. TFA/DCM/0 °C (65%). For **55i**: 1*H*-pyrrole-1-carboxamidine hydrochloride/*i*-Pr_2NEt/DMF/rt (97%).

Within these compounds, significant SAR can be extracted. Various substitutions on the phenyl ring are tolerated, as many potent compounds against *S. aureus* (Table 1) and Gram-positive organisms were obtained. Bulky substituents are detrimental (cf. **20**), and mono- and di-substituted analogs are preferred over tri-substituted derivatives. In general, introduction of heteroaryl groups gave potent compounds against staphylococcal strains. Only **38** and **51** were inactive.

H. influenzae activity was obtained with a few aryl analogs: 4-fluoro (**11**), 2,4-difluoro (**12**), 4-CO_2H (**27**), 4-CN (**25**), 4-$COCH_3$ (**31**) and 3-NH_2 (**22**), but combining



Scheme 4. Alternate synthesis of fully reduced isoxazolinone analogs. Reagents and conditions: (a) $PdCl_2(dppf)/K_3PO_4$/dioxane/80 °C: 71% for **58a**, 97% for **58b**; (b) H_2 (30 psi)/10% Pd–C/EtOH: 97% for **59a**, 96% for **59b**; (c) 1. NaH/EtOCHO, 2. NH_2OH/MeOH/↑↓, 3. $AcOCH_2NHAc/K_2CO_3/CH_2Cl_2$: 31% for **60a**, 38% for **60b**; (d) TFA/CH_2Cl_2 (89%); (e) For **60d**: Ac_2O/Py/0 °C (48%). For **60e**: 1. $TBSOCH_2COCl/Et_3N$/DCM/DMF (79%), 2. TFA/DCM/0 °C (78%).

Table 1. Minimum inhibitory concentration values for isoxazolinone analogs

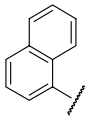
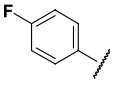
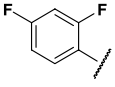
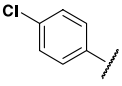
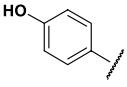
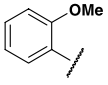
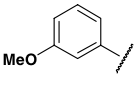
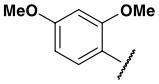
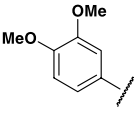
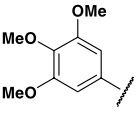
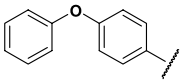
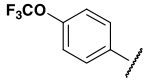
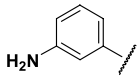
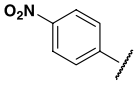
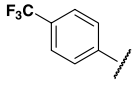
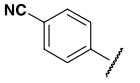
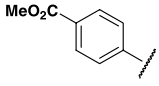
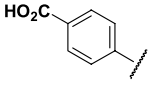
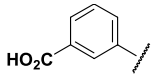
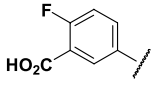
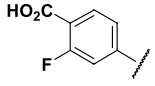
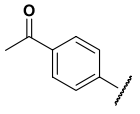
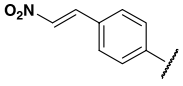
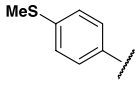
Compd	Structure	MIC[a]
10		1 (32)/>32
11		0.03 (4)/2
12		0.125 (4)/4
13		0.06 (8)/>32
14		1 (2)/8
15		0.5 (8)/32
16		0.06 (1)/>64
17		2 (8)/>32
18		0.25 (1)/32
19		4 (16)/>32

Table 1 (*continued*)

Compd	Structure	MIC[a]
20		64 (>32)/>32
21		0.5 (16)/>32
22		0.125 (2)/2
23		0.03 (1)/>32
24		0.25 (8)/>32
25		0.06 (2)/4
26		0.06 (0.5)/>32
27		1 (8)/2
28		16 (16)/8
29		32(>64)/64
30		4 (32)/32
31		0.06 (2)/4
32		0.125 (4)/4
33		0.5 (16)/>32
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35		0.5 (4)/2
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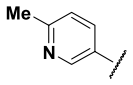
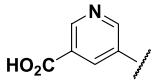
Table 1 (*continued*)

Compd	Structure	MIC[a]
37		0.25 (2)/8
38		32 (>64)/64
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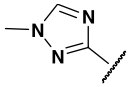
41		4 (8)/>64
42		2 (8)/>64
43		0.5 (4)/4
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45		0.125 (4)/4
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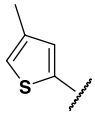
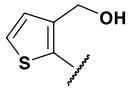
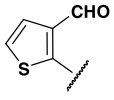
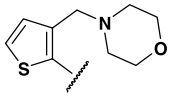
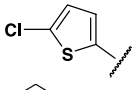
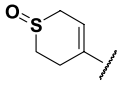
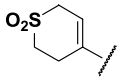
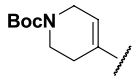
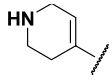
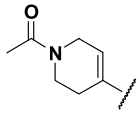
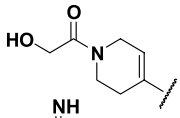
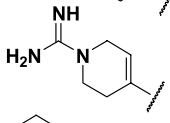
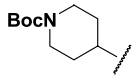
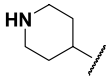
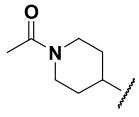
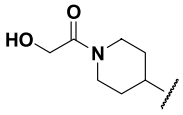
48		0.25 (4)/>64
49		0.25 (4)/32
50		0.5 (1)/64
51		8 (>64)/>64
52		0.06 (8)/4
55a		0.25(1)/4
55b		1(4)/32

Table 1 (*continued*)

Compd	Structure	MIC[a]
55d		0.5(1)/2
55e		0.5(1)/4
55c		8(32)/>64
55f		8(8)/16
55g		0.25(0.5)/2
55h		0.25(0.5)/2
55i		8(16)/32
60a		1(2)/32
60b		8(16)/32
60c		32(16)/32
60d		1(1)/8
60e		1(2)/16
3	Linezolid	1(2)/32

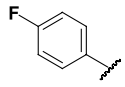
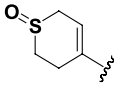
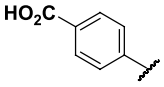
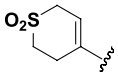
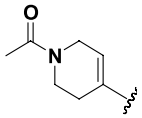
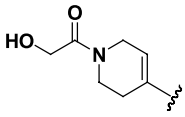
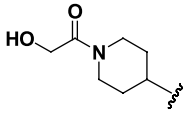
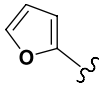
[a] MIC = Minimum inhibitory concentration for the strains and in the format: *S. aureus* (+10% calf serum)/*H. influenzae* (μg/mL).

these substituents was detrimental (cf. **29** and **30**). In vivo efficacy was assessed for **11** and **27** (Table 2), but only **11** was found to be as active as linezolid in vivo.

The pyridines (**34**–**37**) were found particularly active against both *S. aureus* (and other Gram-positive, data not shown) and *H. influenzae* strains. Some of them also showed good in vivo efficacy (see **35** and **36**, Table 2).

The pyrimidine **39** is also worthy of note for its antibacterial activity. It however showed borderline efficacy. The thiazole, furan, and thiophene analogs (**43**, **45**,

Table 2. Mouse in vivo efficacy (PD_{50}) and pharmacokinetics (PK) for selected analogs

Compd	Structure	Miscellaneous[a]	Compd	Structure	Miscellaneous[a]
11		PD_{50} <5 mg/kg/day	**55d**		PD_{50} = 5.6 mg/kg/day PK: C_{max} = 8.3 μg/mL $T_{1/2}$ = 22.2 min AUC = 5.5 μg h/mL
27		PD_{50} >50 mg/kg/day PK: C_{max} = 1.7 μg/mL $T_{1/2}$ = 8.5 min AUC = 0.56 μg h/mL	**55e**		PD_{50} = 4 mg/kg/day PK: C_{max} = 17.7 μg/mL $T_{1/2}$ = 12.2 min AUC = 19.4 μg h/mL
35		PD_{50} = 10 mg/kg/day PK: C_{max} = 15.3 μg/mL $T_{1/2}$ = 86.7 min AUC = 41.1 μg h/mL	**55g**		PD_{50} = 9 mg/kg/day PK: C_{max} = 2.6 μg/mL $T_{1/2}$ = 6.4 min AUC = 1.5 μg h/mL
36		PD_{50} = 8.9 mg/kg/day PK: C_{max} = 17.7 μg/mL $T_{1/2}$ = 203 min AUC = 56 μg h/mL	**55h**		PD_{50} = 4 mg/kg/day PK: C_{max} = 22.9 μg/mL $T_{1/2}$ = 23.5 min AUC = 16.9 μg h/mL
39		PD_{50} = 14.1 mg/kg/day PK: C_{max} = 10.4 μg/mL $T_{1/2}$ = 103.2 min AUC = 29.1 μg h/mL	**60e**		PD_{50} = 3.5 mg/kg/day
45		PD_{50} >50 mg/kg/day	**3**	Linezolid	PD_{50} = 5–6 mg/kg/day[b]

[a] PD_{50} = efficacy evaluation against *S. aureus* in an experimental systemic infection model in mice. Inoculum 1.05×10^7 cfu/mouse i.p. in 7% mucin. Drugs dissolved in 10% DMSO, 5%Tw-80, and water, administered orally b.i.d., 1 and 5 h p.i. Death recorded for 8 days (10 mice per group).
[b] Vehicle = water.

and **47**) also showed broad-spectrum antibacterial activity, but suffered from either CYP liabilities (data not shown) or lack of efficacy (e.g., **45**, Table 2).

In the reduced heterocycle series, the dihydropyran **55a** showed encouraging *H. influenzae* potency. Tetrahydropyran **60a** (the reduced analog of **55a**) displayed slightly reduced potency versus all strains, while the dihydrothiopyran **55b** showed lower potency than dihydropyran **55a**. Oxidation to the sulfoxide **55d** and sulfone **55e** resulted in analogs displaying increased potency and modest mouse PK. Moreover, **55d** showed some improvement from linezolid in a 7-day rat toxicity assay.[14]

In the dehydropiperidine series, **55h** showed good potency, including *H. influenzae*, good PD_{50} and modest mouse PK. In a rat PK study, the half-life ($T_{1/2}$ = 0.6 h) and maximum serum concentration (C_{max} (oral) = 1.9 mg/mL) were less than stellar. Moreover, testing in a 7-day rat toxicity assay did not show any advantage over linezolid.[14] The piperidine series showed decreased potency over their corresponding dehydro analogs. However, the hydroxyacetamides **55h** and **60e** showed similar oral efficacy.

In conclusion, the synthesis of C-linked derivatives was shown to be straightforward from late stage intermediates through different cross-coupling approaches. Most of the compounds were found to be potent against Gram-positive strains. Some were also found to have broad-spectrum antibacterial activity: **22** (3-NH_2-phenyl), **27** (4-CO_2H-phenyl), **39** (4-pyrimidinyl), **47** (3-thienyl), **55h** (tetrahydropyridine), and **55d** (dihydrothiopyran-sulfoxide). The sulfoxide **55d** was shown to be slightly less toxic than linezolid in a 7-day rat toxicity assay.

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